Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Epicardial adipose tissue, inflammatory biomarkers and COVID-19: Is there a possible relationship?

Alireza Abrishami [a], Vahid Eslami [b,c,*], Zahra Baharvand [a], Nastaran Khalili [d,e], Somayeh Saghamanesh [f], Ehsan Zarei [a], Morteza Sanei-Taheri [g,h]

[a] Department of Radiology, Shahid Labbafinejad Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Department of Cardiology, Shahid Labbafinejad Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[c] Cardiovascular Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[d] School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[e] Cancer Immunology Project, Universal Scientific Education and Research Network (USERN), Tehran, Iran
[f] Center for X-ray Analytics, Empa, Swiss Federal Laboratories for Materials Science and Technology, 8600 Dubendorf, Switzerland
[g] Iranian Society of Radiology, Tehran, Iran
[h] Department of Radiology, Shohada-E-Tajrish Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background & Aims: Adipose tissue is a biologically active organ with pro-immunogenic properties. We aimed to evaluate the prognostic value of epicardial adipose tissue (EAT) in COVID-19 and its correlation with other inflammatory biomarkers.

Material and Methods: One-hundred patients with COVID-19 were enrolled. C-reactive protein (CRP), lactate dehydrogenase (LDH), neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-CRP ratio (LCR), and platelet-to-lymphocyte ratio (PLR) were evaluated on admission. EAT volume and density were measured by computed tomography. Patients were followed until death or discharge. Univariate and multivariate analysis was performed and ROC curve analysis was used to assess the ability of inflammatory markers in predicting survival. The relationship between EAT and other inflammatory markers was also investigated.

Results: The mean ± SD age of patients was 55.5 ± 15.2 years old; 68% were male. Univariate analysis revealed that increased lung involvement, blood urea nitrogen, LDH and NLR, and decreased platelet count were significantly associated with death. After adjustment, LDH was independently predictive of death (OR = 1.013, p-value = 0.03). Among inflammatory markers, LCR had the best ability for predicting survival with 79.7% sensitivity and 64.3% specificity at an optimal cut-off value of 20.8 (AUC = 0.744, 95% CI = 0.612–0.876, p-value = 0.004). EAT volume demonstrated positive correlation with NLR and PLR (p = 0.001 and 0.01), and a negative correlation with LCR (p = 0.02). EAT density was significantly different between decedents and survivors (p = 0.008).

Conclusion: Routine laboratory tests that represent status of inflammation can be used as cost-effective prognostic markers of COVID-19. Also, the significant association between EAT volume and other inflammatory biomarkers might explain the more severe disease in obese patients.

1. Introduction

Coronavirus disease 2019 (COVID-19) is a complex multi-system infectious disease that predominantly affects the lungs [1]. From when it was first identified in December 2019, COVID-19 has caused significant burden on healthcare systems worldwide. As this pandemic continues to threaten global health, it is essential to further expand our knowledge about factors that can affect survival. Besides, increasing our understanding of different pathways that are involved in the pathogenesis and clinical severity of COVID-19 can help identify possible therapeutic targets and improve clinical management.

Inflammation plays a major role in the development and progression of COVID-19 [2]. Already, several studies have demonstrated the value of inflammatory biomarkers such as C-reactive protein (CRP) and serum

* Corresponding author at: Department of Cardiology, Shahid Labbafinejad Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
E-mail address: veslami59@gmail.com (V. Eslami).

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lactate dehydrogenase (LDH) in predicting the clinical severity of COVID-19 [3–5]. Therefore, factors that convey information about the status of inflammation in patients with COVID-19 can be beneficial in predicting the course of disease and determining prognosis.

Based on growing evidence, obesity, in particular visceral adiposity, has an undeniable role in adversely affecting the course of COVID-19 and increasing the risk of mortality [6]. Obesity is associated with a chronic state of inflammation that, not only leads to metabolic disturbances, but also alters adaptive and innate immune responses [7]. In a recent study, Ryan and colleagues proposed that the voluminous and hyper-vascularized epicardial adipose tissue (EAT) in obese patients functions as a reservoir of COVID-19, facilitating viral spread and augmenting immune response. They stated that the subsequent activation of the cytokine cascade and enhanced production of pro-inflammatory cytokines such as interleukin-6 could be responsible for the unfavorable disease outcome in patients with obesity [8]. Thus, it is probable that therapies which target adipose tissue could help reduce the burden of COVID-19.

Considering these findings, we aimed to investigate the association of EAT volume and density with the prognosis of patients with COVID-19. Furthermore, we hypothesized that EAT volume and density may be correlated with other inflammatory biomarkers such as CRP, LDH, neutrophil-to-lymphocyte-ratio (NLR), platelet-to-lymphocyte-ratio (PLR) and lymphocyte-to-CRP ratio (LCR) in patients with COVID-19. We hope that the findings of this study will provide insight into the identification of novel prognostic markers and the development of rational therapeutic strategies.

2. Material and methods

2.1. Ethics approval and consent to participate

The ethical review board of our institution approved the study. Written informed consent was obtained from all individuals prior to being enrolled in the study. All investigations were conducted in accordance with the 1964 Declaration of Helsinki and its later amendments.

2.2. Study design and patient population

This single-center study was conducted on 100 patients with a confirmed diagnosis of COVID-19 who were admitted to our academic tertiary hospital from 20 February 2020 to 20 April 2020. Inclusion criteria were as follows: 1) confirmed diagnosis of COVID-19 through real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay with samples obtained from a nasopharyngeal swab and 2) chest computed tomography (CT) imaging suggestive for COVID-19 pneumonia. Exclusion criteria were as follows: 1) poor or suboptimal image quality due to severe cardiac motion artifact; 2) age less than 18 years, and; 3) history of prolonged steroid and NSAID drug use.

On admission, patients' information including demographic data, clinical characteristics and laboratory findings were collected. The imaging findings of patients' initial chest CT were also recorded. All patients were followed until one of the study endpoints (defined as either death or complete recovery and discharge) was reached.

2.3. Laboratory procedures

RT-PCR for SARS-CoV-2 (DAAN gene Co Ltd device) was performed on the nasopharyngeal swab specimen of all patients. Laboratory tests including serum biochemistry, complete blood count (CBC) and inflammatory markers such as CRP, LDH and creatine phosphokinase (CPK) were recorded. NLR, PLR and LCR were also calculated for each individual by using CBC findings. CRP levels were measured using the Rondox essay kit with immunoturbidimetric techniques. For the assessment of CBC, venous blood samples were collected in potassium ethylenediaminetetraacetic acid tubes (dipotassium EDTA tubes) and the Sysmex-XE 2000i automated blood cell analyzer (Sysmex, Kobe, Japan) was used for measurement within an hour. This is the standard duration time for our laboratory since it prevents EDTA-induced swelling.

2.4. Chest CT imaging

As part of national COVID-19 guidelines, all clinically suspected patients underwent low-dose non-enhanced chest CT at admission [9]. In patients with more than one CT, only the initial CT was evaluated. CT was performed for every patient using a 64-slice scanner (Siemens sensation; Siemens Healthineers, Erlangen, Germany) in a supine position during end-inspiration. A low-dose CT protocol with the following scanning parameters was applied: gantry rotation time of 0.5 s, 0.625 mm × 64-detector array, pitch of 1.4, table speed of 45.2 mm/rotation, 20 mAs, 120 kVp, and 300 × 300 matrices. CARE Dose4D and CARE kV scanning parameters were off. A 1 mm slice thickness and 1 mm reconstruction interval were used for sagittal and coronal image reconstruction. After each CT, passive air ventilation was performed for at least 30 min and machine surfaces were disinfected with ethanol and didecyldimethylammonium chloride (DDAC).

DICOM data was transferred onto a picture archiving and communicating system (PACS). Then, two expert radiologists with 9 and 18 years of experience independently interpreted the images and a final decision was reached by consensus. Both radiologists were blinded to laboratory data, clinical features, and patients' diagnosis. In case of disagreement, the opinion of a third radiologist was used. All CT images were viewed in axial, sagittal and coronal planes. The predominant pattern of involvement was classified as ground-glass opacification, consolidation, reticular or mixed. In addition, axial and anteroposterior lesion distribution were also recorded. The presence of other imaging features such as pleural and pericardial effusion, crazy-paving pattern, reversed halo sign, airway thickening, vessel dilatation, airway dilatation, air bronchogram and lymphadenopathy (defined as a lymph node with a short-axis diameter >10 mm) was also assessed. Also, all images were reviewed for the presence or absence of coronary artery calcification (CAC) in each of the following vessels: (1) left main or left anterior descending coronary artery and its branches, (2) left circumflex coronary artery and branches, and (3) right coronary artery. For evaluation of zonal involvement, three zones were defined as follows: (1) upper zone: area above the carina region; (2) middle zone: area between the carina and inferior pulmonary vein; and (3) lower zone: area below the inferior pulmonary vein. The percentage of lung involvement was scored using the following system: 0: no involvement, 1: <25%, 2: 26–50%, 3: 51–75% and 4: >75%. Zonal scores of both lungs were summed up to calculate upper, middle, and lower zone score (maximum score of each zone = 8). The total lung score was calculated by summing scores of all of the three zones (maximum score = 24).

All dimensions of cardiac indices were derived from patients' chest CT scan. Epicardial fat was defined as the adipose tissue between the visceral epicardium to the outside of the myocardium. The open-source 3D Slicer software (version 14.10.2) on Microsoft Windows was employed to segment and analyze the intended volumes in each patient (the 3D Slicer software is a platform for the analysis and visualization of medical images, which supports multimodal imaging like CT, magnetic resonance imaging (MRI), ultrasound, nuclear medicine, and microscopy, and is capable of interactive segmentation, registration, and volume rendering [10]). Subsequently, CT volumes (in DICOM format) were loaded in the software. The epicardial fat was segmented in an embedded segment editor module in the relevant slices of each CT volume. All segments were analyzed through the quantification platform in the software. For this purpose, a segment statistics module was used to compute specific segment volumes and density based on the Hounsfield Unit (HU) scale (Fig. 1). A script code was written in Matlab software to read all of the obtained 3D Slicer data files and organize the

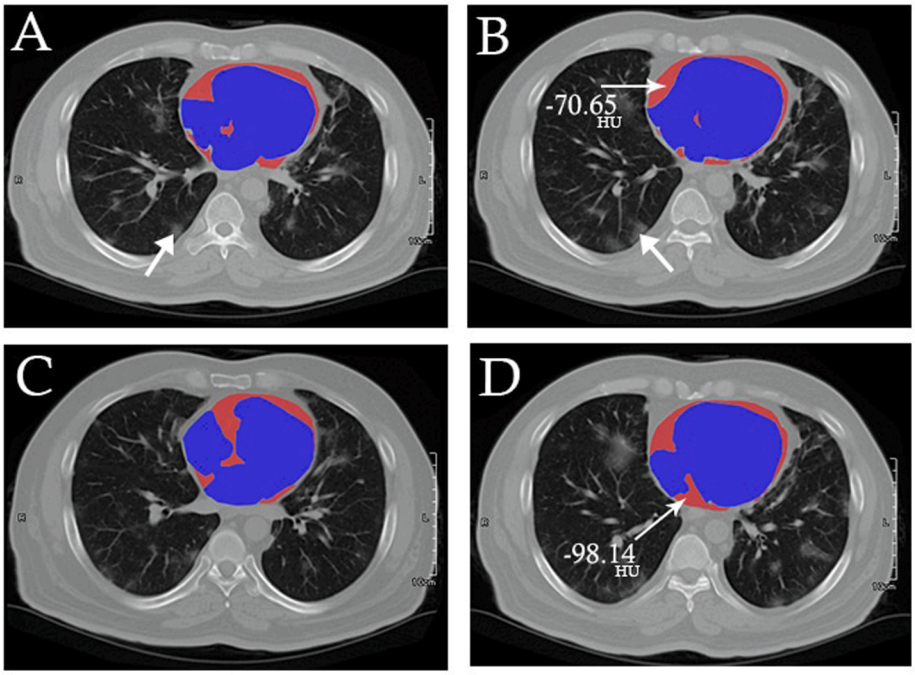

Fig. 1. Quantification of epicardial adipose tissue density by CT scan.

measurements related to each patient.

2.5. Statistical analysis

Categorical variables are reported as frequency (percentage). Normal continuous variables are expressed as mean (standard deviation (SD)) and skewed continuous variables are presented as median and (Quartile 1- Quartile 3). All measurements were assessed with normality tests. Continuous data were compared by using the Student *t*-test or Mann-Whitney *U* test and the Chi-square test or Fisher's exact test was applied for the comparison of categorical variables. Univariate and multivariate logistic regression analyses (using backward method) were performed to identify predictive factors of COVID-19-related death and Spearman's correlation test was used to evaluate the relationship between inflammatory markers and EAT volume and density. Furthermore, receiver operator characteristic (ROC) curve analysis was performed to calculate the optimal threshold value of inflammatory markers and their specificity and sensitivity for distinguishing survivors from non-survivors. All statistical tests were performed by SPSS version 23 (IBM Inc., Chicago, IL, USA). A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Demographic, clinical and imaging findings

Table 1 shows patients' baseline demographic and clinical data. The mean ± SD age of patients was 55.5 ± 15.2 years old; 68% were male. The most common presenting symptoms were cough and dyspnea, observed in 68% and 65% of patients, respectively. On follow-up, 17 (17%) patients died and the remaining were discharged. The mean hospitalization time was significantly longer in deceased patients compared with those who were discharged (16.71 ± 6.89 versus 10.41 ± 7.27 days, $p < 0.001$). As shown in Table 1, there was no significant difference regarding the age of deceased and discharged patients ($p = 0.63$); however, there was a significant male predominance in decedents ($p = 0.04$). Furthermore, comorbidities such as hypertension ($p = 0.04$), obesity ($p = 0.02$) and presence of immunocompromised conditions ($p = 0.008$) were significantly more prevalent among patients who died.

Evaluation of laboratory parameters indicated that the median platelet count was significantly lower among deceased patients ($p = 0.038$); however, no significant difference was seen across the two groups in terms of leukocyte, lymphocyte and eosinophil count (Table 1). Lymphopenia, which was defined as a lymphocyte count of less than 1×10^9/L, was observed in 30% of the study cohort. Renal function markers such as serum creatinine ($p < 0.001$) and BUN ($p < 0.001$), and inflammatory markers such as serum LDH ($p < 0.001$) and NLR ($p = 0.025$) displayed significantly higher levels in patients who died. LCR was significantly lower in non-survivors ($p = 0.004$).

Table 2 shows the imaging finding of patients on admission. The majority of patients presented with bilateral lesions (87%) with peripheral (74%) and posterior distribution (70%). Ground-glass opacification was the most frequently observed infiltration pattern ($n = 66$) followed by consolidation ($n = 16$). There was no significant difference between the survivors and non-survivors in terms of their initial presenting pattern. Regarding other abnormal imaging features, airway thickening (74%) and vessel dilatation (68%) were the most prevalent findings in the study cohort; vessel dilatation was present in all of the patients who died. On the other hand, cavitation was absent in the entire cohort and emphysema, fibrosis, and bronchiectasis were seen in only one patient each. Our results did not display a significant difference in CAC between the discharged and deceased groups. Eighteen patients (18%) including 13 patients in the discharged group and five in the deceased group had pleural effusion at presentation, of which in 55.5% of cases, this effusion was bilateral. As shown by the higher CT scores, the extent of lung involvement was significantly greater in those who died ($p < 0.001$). The median epicardial fat volume was 72.97 ml (Q1-Q3: 50.2–109.03) and the median epicardial fat density was −92.5 HU (Q1-Q3: −108.1 to −76.6); the values for EAT volume were not significantly different among deceased and survived patients but there was a marked difference in terms of EAT density ($p = 0.79$ and $p = 0.008$, respectively).

3.2. Predictors of mortality based on logistic regression analysis

Univariate analysis revealed that higher total lung score, increased

Table 1
Patients' baseline demographic, clinical and laboratory characteristics.

	Total (n = 100)	Discharged (n = 83)	Deceased (n = 17)	p-value
Age(years)	55.5 ± 15.2	55.2 ± 15.6	57.1 ± 13.2	0.637
Sex				
Male	68 (68)	53 (63.8)	15 (88.2)	0.04
Female	32 (32)	30 (36.1)	2 (11.8)	
Hospitalization time (day)	11.49 ± 7.56	10.42 7.27	16.71 6.89	<0.001
Presenting signs and symptoms				
Fever	63 (63)	53 (63.8)	10 (58.8)	0.69
Cough	68 (68)	57 (68.7)	11 (64.7)	0.79
Sore throat	12 (12)	11 (13.2)	1 (5.8)	0.68
Dyspnea	65 (65)	54 (65.1)	11 (64.7)	0.98
Chilling	20 (20)	19 (22.9)	1 (5.8)	0.18
Headache	16 (16)	15 (18.1)	1 (5.8)	0.29
Nausea	11 (11)	10 (12.1)	1 (5.8)	0.68
Abdominal pain	12 (12)	10 (12.1)	2 (11.7)	>0.999
Diarrhea	10 (10)	8 (9.6)	2 (11.7)	0.68
Myalgia	30 (30)	27 (32.5)	3 (17.6)	0.22
Presence of comorbidities				
Asthma	8 (8)	7 (8.4)	1 (5.8)	0.71
Diabetes	21 (21)	20 (24.1)	1 (5.8)	0.11
Cardiovascular disease	21 (21)	16 (19.3)	5 (29.5)	0.34
Hypertension	33 (33)	31 (37.3)	2 (11.7)	0.04
Chronic kidney disease	22 (22)	15 (18.1)	7 (4.1)	0.05
Chronic liver disease	1 (1)	1 (1.2)	0 (0)	>0.999
COPD	1 (1)	1 (1.2)	0 (0)	>0.999
Immunodeficient state [a]	13 (13)	7 (8.4)	6 (35.3)	0.008
Obesity (BMI ≥ 30)	25 (25)	17 (20.5)	8 (47.1)	0.02
Oxygen saturation (%)	90 (87-93)	90 (87-93)	88 (87-90)	0.11
Type of ventilation				
None	80 (80)	80 (96.4)	0 (0)	<0.001
BiPAP	2 (2)	1 (1.2)	1 (5.8)	
Intubation	18 (18)	2 (2.4)	16 (94.1)	
Complete blood count				
Leukocyte ($\times 10^9$ /L)	6.02 ± 2.63	5. 89 ± 2.62	6.68 ± 2.67	0.27
Neutrophil ($\times 10^9$ /L)	4.47 ± 2.47	4.29 ± 2.41	5.37 ± 2.67	0.11
Lymphocyte ($\times 10^9$ /L)	1.16 (0.89–1.57)	1.15 (0.87–1.49)	1.02 (0.68–1.32)	0.11
Eosinophil ($\times 10^9$ /L)	0.20 (0.10–0.20)	0.20 (0.10–0.20)	0.15 (0.10–0.20)	0.65
Hemoglobin (g/dL)	13.56 ± 2.50	13.73 ± 2.51	12.71 ± 2.36	0.14
Platelet ($\times 10^9$ /L)	198 (145–253)	203 (157–263)	166 (128.5–208)	0.04
Inflammatory markers				
LDH (IU/l)	416 (336.5–522.5)	363 (332–470)	578 (473–931)	<0.001
CRP (mg/dL)	34.73 ± 20.89	32.96 ± 21.06	44.18 ± 17.74	0.065
CPK (IU/l)	99 (58–190)	99 (56–167)	114 (82–331)	0.41
NLR	3.39 (2.37–4.97)	3.02 (2.29–4.37)	5.02 (2.8–10.4)	0.02
LCR	31.9 (19.6–63.9)	33.9 (22.8–85)	17.2 (14.3–28.9)	0.004
PLR	162.8 (124.2–222.4)	160.8 (124.2–219.4)	202.0 (120.7–201.2)	0.46
Blood urea nitrogen (mg/dL)	30 (18.5–45)	27.5 (17–40)	46 (37–124)	<0.001
Serum creatinine (mg/dL)	1.26 (1.02–1.67)	1.18 (1.00–1.38)	1.77 (1.43–3.08)	<0.001
Troponin (ng/ml)	0.002 (0.001–0.007)	0.002 (0.001–0.005)	0.006 (0.002–0.012)	0.068

[a] Consists of organ transplant recipients or patients with active malignancy who were receiving immunosuppressive treatment at the time of the study.
Abbreviations: NLR: neutrophil-to-lymphocyte ratio; LCR: lymphocyte-to- CRP ratio; CRP: C-reactive protein; LDH: lactate dehydrogenase; CPK: Creatinephosphokinase; COPD: Chronic obstructive pulmonary disease.

Table 2
Patients' imaging findings on initial CT scan.

	Total (n = 100)	Discharged (n = 83)	Deceased (n = 17)	p–value
Lung involvement CT score				
Upper	2.0 (1.0–4.0)	2.0 (0.0–3.0)	4.0 (2.0–5.0)	0.002
Middle	3.0 (2.0–6.0)	3.0 (2.0–5.0)	6.0 (5.0–7.0)	<0.001
Lower	4.0 (2.0–6.0)	4.0 (2.0–5.0)	7.0 (5.0–8.0)	<0.001
Total	9.0 (5.0–15.0)	8.0 (4.0–14.0)	16.0 (13.0–19.0)	<0.001
Epicardial fat volume (ml)	73.0 (50.2–109.1)	75.8 (50.5–108.3)	63.0 (50.0–115.1)	0.794
Epicardial fat density (Hounsfield unit)	−92.5 (−108.1 to −76.6)	−89.1 (−105.0 to −75.0)	−102.9 (−115.0 to −83.0)	0.008
Involvement pattern				
Ground glass opacification	66 (66)	57 (68.7)	9 (52.9)	
Consolidation	18 (18)	12 (14.4)	6 (35.3)	
Reticular	8 (8)	8 (9.6)	0 (0)	
Mixed	8 (8)	6 (7.2)	2 (11.7)	
Lesion Distribution				
Axial				
Peripheral	74 (74)	63 (75.9)	11 (64.7)	
Central	8 (8)	7 (8.4)	1 (5.8)	0.42
Diffuse	18 (18)	13 (15.6)	5 (29.5)	
Craniocaudal				
Upper	10 (10)	9 (10.8)	1 (5.8)	
Middle	14 (14)	13 (15.6)	1 (5.8)	0.46
Lower	47 (47)	40 (48.2)	7 (41.1)	
Diffuse	29 (29)	21 (25.3)	8 (47.1)	
Anteroposterior				
Anterior	9 (9)	6 (7.2)	3 (17.6)	
Posterior	70 (70)	61 (73.5)	9 (52.9)	0.16
Diffuse	21 (21)	16 (19.2)	5 (29.5)	
Bilateral	87 (87)	70 (84.3)	17 (100)	
Unilateral	13 (13)	13 (15.6)	0 (0)	0.12
Other abnormal findings				
Airway thickening	77 (77)	61 (73.5)	16 (94.1)	0.11
Dilated vessel	68 (68)	51 (61.4)	17 (100)	<0.001
Airway dilatation	41 (41)	30 (36.1)	11 (64.7)	0.03
Septal thickening	13 (13)	9 (10.8)	4 (23.5)	0.23
Pleural effusion	18 (18)	13 (15.6)	5 (29.4)	0.05
Pericardial effusion	15 (15)	12 (14.4)	3 (17.6)	0.72
Coronary artery calcification	47 (47)	38 (45.8)	9 (52.9)	0.49
Emphysema	1 (1)	1 (1.2)	–	>0.999
Bronchiectasis	1 (1)	1 (1.2)	–	>0.999
Fibrosis	1 (1)	1 (1.2)	–	>0.999
Crazy paving	15 (15)	10 (12)	5 (29.4)	0.13
Reversed–halo	1 (1)	1 (1.2)	–	>0.999
Air bronchogram	36 (36)	26 (31.3)	10 (58.8)	0.03
Cavitation	–	–	–	–
Cyst	11 (11)	11 (13.2)	–	0.20
Lymphadenopathy	4 (4)	3 (3.6)	1 (5.8)	0.53

BUN, LDH and NLR, and decreased platelet count were significantly related to the death of patients with COVID-19. After performing multivariate analysis and adjusting for variables, baseline LDH at admission was an independent predictor of COVID-19-related death (adjusted OR = 1.013, 95% CI: 1.00–1.02, p-value = 0.03) (Table 3).

Table 3
Univariate and multivariate logistic regression analysis showing predictive factors of COVID-19-related death.

	Crude OR	95% CI for OR		p-value	Adjusted OR[a]	95% CI for OR		p-value
		Lower	Upper			Lower	Upper	
Total Lung score	1.215	1.093	1.35	<0.001	–	–	–	–
Serum Creatinine	1.368	0.99	1.89	0.056	–	–	–	–
Blood urea nitrogen	1.019	1.01	1.03	0.005	1.047	0.99	1.10	0.088
Platelet count	0.992	0.98	1.00	0.041	0.976	0.94	1.01	0.141
NLR	1.124	1.01	1.25	0.036	–	–	–	–
CRP	1.028	0.99	1.06	0.071	–	–	–	–
LDH	1.003	1.00	1.01	0.048	1.013	1.00	1.02	0.037
LCR	0.985	0.96	1.01	0.985	–	–	–	–
EAT volume	1.006	0.99	1.01	0.265	–	–	–	–
EAT density	0.985	0.96	1.00	0.148	0.91	0.81	1.02	0.10

[a] Backward elimination approach was used. Empty cells represent the variables which have been omitted from the model in backward method. **Abbreviations:** NLR: neutrophil-to-lymphocyte ratio; LCR: lymphocyte-to- CRP ratio; CRP: C-reactive protein; LDH: lactate dehydrogenase; EAT: epicardial adipose tissue

3.3. ROC curve analysis of inflammatory biomarkers for predicting survival

Fig. 2 depicts the ROC curve of LCR. As shown, the optimal cut-off value of LCR for separating survivors from non-survivors was 20.8 with 79.7% sensitivity and 64.3% specificity (AUC = 0.744, 95% CI = 0.612–0.876, p-value = 0.004). Also, the optimal threshold of NLR for differentiating survival from death was 3.65 with 62.5% sensitivity and 60% specificity (AUC = 0.678, 95% CI = 0.512–0. 84, p-value = 0.08) (Fig. 3). The AUC for PLR was 0.559 (95% CI = 0.368–0.731, p-value = 0.455); however, an optimal cut-off point with reasonable sensitivity and specificity was not found (Fig. 4).

3.4. Association between EAT volume and density with inflammatory markers

As shown in Table 4, EAT volume had a significant positive association with NLR (r = 0.33, p = 0.001) and PLR (r = 0.25, p = 0.01). Also, increased EAT volume showed a significant but mild correlation with decreased values of LCR (r = −0.25, p = 0.02). However, no significant association was seen between EAT density and NLR, PLR, or LCR (p = 0.35, p = 0.58, p = 0.69, respectively).

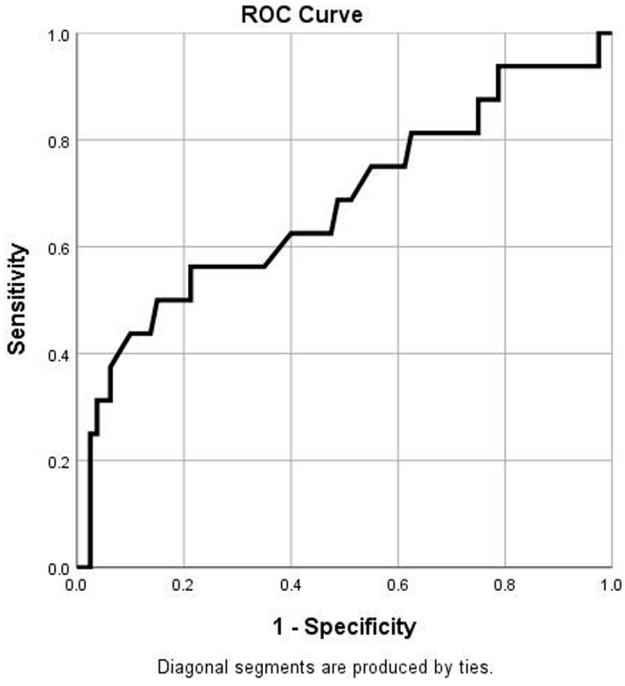

Fig. 2. Receiver operating characteristic (ROC) curve analysis of neutrophil-to-lymphocyte ratio for predicting survival.

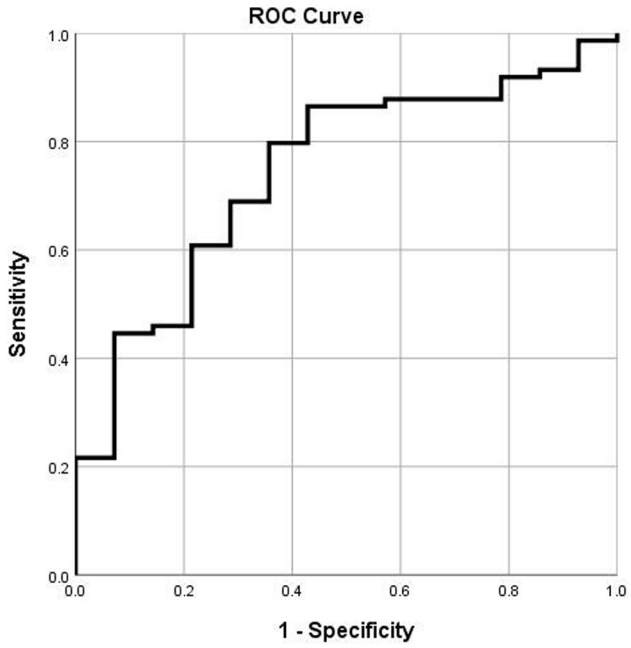

Fig. 3. Receiver operating characteristic (ROC) curve analysis of lymphocyte-to-CRP ratio for predicting survival.

4. Discussion

Inflammation plays a major role in the pathogenesis and prognosis of COVID-19 [2]. This finding has triggered many studies to focus on investigating cost-effective and easily accessible inflammatory biomarkers such as CRP, LDH, LCR, NLR and recently, EAT, for predicting the prognosis of patients with COVID-19. In this study, we showed that serum LDH can be used as a single biomarker to independently predict COVID-19-related death. Furthermore, we observed that LCR and NLR have acceptable sensitivity and specificity for separating survivors from non-survivors at an optimal cut-off value of 20.8 and 3.65, respectively. Our findings were also indicative of a significant difference in the EAT attenuation values of decedents compared with survivors. In addition, EAT volume demonstrated significant but mild positive associations with NLR and PLR, and a negative correlation with LCR.

Severe infections result in cytokine-mediated tissue injury and release of LDH [11]. LDH is largely present in the lung tissue; thus, as with other respiratory infections such as the Middle East Respiratory Syndrome (MERS), patients with severe COVID-19 are also likely to have increased amounts of LDH in their circulation [12]. Several studies have

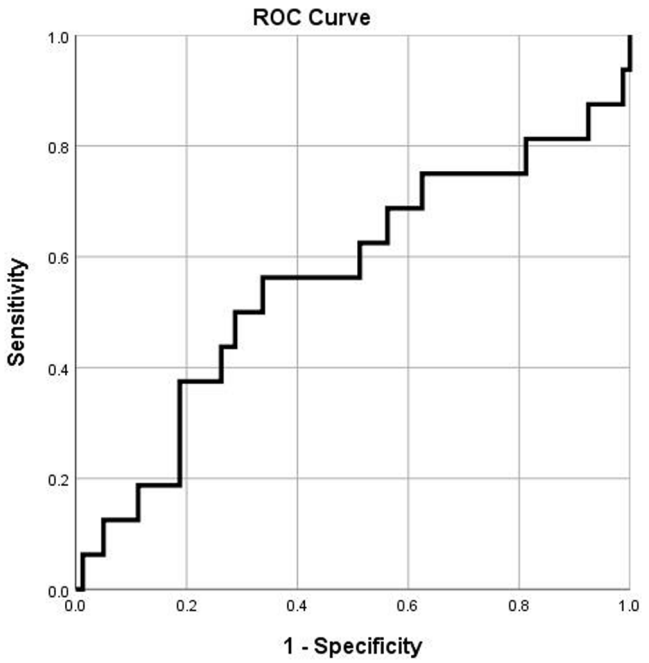

Fig. 4. Receiver operating characteristic (ROC) curve analysis of platelet-to-lymphocyte ratio for predicting survival.

Table 4
Correlation analysis to evaluate the association between epicardial adipose tissue and inflammatory markers.

	EAT volume		EAT density	
	Correlation coefficient (r)[a]	p-value	Correlation coefficient (r)[a]	p-value
NLR	0.33	0.001	−0.09	0.351
PLR	0.25	0.01	−0.06	0.58
LCR	− 0.25	0.02	0.04	0.69

[a] Calculated by Spearman's correlation test. **Abbreviations:** NLR: neutrophil-to-lymphocyte ratio; LCR: lymphocyte-to- CRP ratio; CRP: C-reactive protein; PLR: platelet-to-lymphocyte ratio; EAT: epicardial adipose tissue.

reported an association between elevated LDH and poor outcome in patients with COVID-19 [1,13,14]. The results of a recent pooled analysis showed that elevated levels of LDH can increase the odds of mortality by more than 16-fold in patients with COVID-19. Moreover, it was shown that increased LDH was present in more than 95% of patients who died while less than 60% of survivors displayed elevated LDH [5]. We also observed that LDH levels are significantly higher in deceased patients and that LDH is an independent predictor of mortality. Based on our results, a one-unit rise in the level of LDH increased the chance of death by approximately 1.3%. Consistent with our findings, in a mortality prediction model of COVID-19, increased LDH was able to independently predict the vast majority of patients requiring special attention and care [15]. Taken together, these findings emphasize the value of LDH as an excellent prognostic marker of COVID-19 that can be used routinely for hospitalized patients.

Based on experience from previous epidemics, laboratory parameters that represent alterations in the hematological system can be useful for assessing prognosis and predicting the risk of mortality [16,17]. Moreover, their cost-effectiveness and feasibility is an asset. Previous studies in patients with MERS-CoV suggested that lymphocytopenia and elevated CRP level are predictive of pneumonia development and its progression into ARDS [17]. Interestingly, increased pro-inflammatory cells and cytokines in the sera of patients with aberrant pulmonary inflammation and extensive lung damage were also reported in SARS-CoV infection [16]. In this aspect, several studies have shown that absolute neutrophil and lymphocyte count, as well as ratios that indicate the proportion of these cells could be used as markers of advanced disease in patients with COVID-19 [18,19]. NLR, PLR and LCR are novel inflammatory markers, which have recently gained attention for predicting survival in a multitude of diseases including COVID-19. These markers are easily accessible through daily-performed laboratory tests such as CBC and CRP. In a study by Cheng et al. that was conducted on 465 patients with COVID-19, NLR was shown to be an effective predictor of disease progression; they showed that at a cut-off value of 3.19, NLR has 78.2% sensitivity and 73.9% specificity for predicting death (AUC = 0.81, 95% CI, 0.73–0.88, $P < 0.001$) [20]. In another study, a similar cut-off value of 3.00 was reported for NLR, which had 100% sensitivity and 73.1% specificity for predicting severe disease [21]. In a recent meta-analysis that aimed to compare NLR and LCR between patients with severe versus non-severe form of COVID-19, NLR values were significantly higher in patients with severe disease (standard mean difference = 2.404, 95% CI = 0.98-3.82) whereas LCR values were markedly lower in these patients (standard mean difference = −0.912, 95% CI = −1.275 to −0.550) [22]. In our study, the cut-off values of NLR and LCR for predicting COVID-19-related death were 3.65 and 20.8, respectively. Moreover, our observation regarding significantly higher values of NLR in patients who experience death was consistent with that of other studies [13,21,23]. Nevertheless, we were not able to demonstrate a significant difference in terms of LCR between discharged and deceased patients.

Besides the importance of neutrophils and lymphocytes, changes in the platelet count can also be indicative of severe COVID-19 [24,25]. PLR is a novel biomarker that can simultaneously reflect the changes in platelet and lymphocyte count. Due to the interactions between platelets and lymphocytes, PLR indicates both aggregation and inflammation and might be a more sensitive marker for the intensity of systemic inflammation rather than platelets or lymphocytes alone. Qu et al. showed that baseline platelet count was lower in patients with severe COVID-19 disease [26].; our study demonstrated the same result, showing significantly lower platelet counts in deceased patients on admission. However, in contrast to our findings, they displayed that PLR was non-significantly lower in severe patients at admission but increased significantly in severe patients compared with non-severe patients during the disease course. They concluded that PLR can change dynamically throughout the treatment course and has the potential to be used as an indicator of disease severity and prognosis in hospitalized patients with COVID-19 [26]. In another report on 93 patients with COVID-19, similar to our study, PLR was found to be significantly higher in severe patients; however, the disease stage at which the PLR values were obtained was not mentioned [15].

Another example of a novel inflammatory biomarker with potential prognostic value is epicardial adipose tissue. It is now evident that adipose tissue is not just a simple deposit for fat storage but rather a biologically active organ with pro-immunogenic properties that can induce acute inflammation in organs such as the heart and vasculature through activation of the immune system [27]. Interestingly, during the H1N1 influenza virus pandemic, obesity was shown to increase the risk of severe infection and death [28]. Moreover, studies suggest that the duration of viral shedding in obese patients infected with influenza is significantly prolonged compared to others [29]. Several recent studies have also indicated a possible association between increased adipose tissue in obese individuals and poor prognosis of COVID-19 [30–32]. As mentioned earlier, Ryan and Caplice hypothesized that adipose tissue can act as a reservoir for extensive SARS-CoV-2 spread, leading to enhanced immune activation and increased cytokine production. They proposed that stimulation of the immune system by pro-inflammatory mediators such as adipokines, which are released from fat tissue, might predispose obese patients infected with COVID-19 to the so-called "systemic cytokine storm" and subsequently result in clinical deterioration and death [8]. Thus, besides being associated with

cardiometabolic conditions such as diabetes and cardiovascular events, increased adipose tissue can independently contribute to an increased risk of COVID-19-related mortality through an inflammatory process. Meanwhile, the epicardial adipose tissue, which directly surrounds the heart and is highly integrated within the coronary vasculature, could be of more interest.

Considering the high spatial resolution of CT and the discrete attenuation values of adipose tissue, EAT volume and density can be easily and precisely measured by CT scan [33]. In our country, from the beginning of the COVID-19 pandemic, chest CT was performed routinely at admission for all patients who presented with high clinical suspicion of COVID-19. This extensive use of CT provided an advantage for the simultaneous evaluation of possible prognostic markers of COVID-19 such as EAT volume and EAT density. In the present study, we showed that CT-measured EAT density was significantly lower in patients who died; however, it failed to show a prognostic value for predicting survival after performing regression analysis. Furthermore, the results of our study did not display a significant difference in EAT volume between survivors and non-survivors. In a recent study by Deng e al., similar to the result of our study, it was reported that in patients with severe COVID-19, EAT density was lower compared with patients who had acquired a mild form of the disease ($p = 0.05$). However, they found that EAT volume was significantly higher in patients with severe COVID-19 [34]. In a retrospective study, Iacobellis et al. also evaluated non-gated CT scans of patients with mild and severe COVID-19 to compare EAT density and thickness measurements among these patients. They showed that EAT thickness was not significantly different among patients with different degrees of COVID-19 severity; however, EAT attenuation increased markedly with an increase in the severity of COVID-19 [35].

We observed a significant association between increased EAT volume and elevated NLR and PLR and decreased LCR. Current evidence supports the finding of our study regarding a correlation between EAT and inflammatory markers in a multitude of diseases. In a recent study, a positive correlation was observed between EAT thickness and NLR and CRP in hypertensive patients [36]. Akil and colleagues also showed a mild correlation between EAT thickness and NLR in patients with cerebral ischemic stroke [37]. Other studies conducted on patients with inflammation-associated diseases such as lichen planus or diabetes mellitus have also demonstrated that EAT thickness is positively correlated with NLR, PLR and CRP [38,39]. Nevertheless, we did not observe a significant association between EAT density and inflammatory biomarkers. Consistent with this finding, Iacobellis and colleagues also did not find EAT density to be significantly correlated with the level of IL-6 [35].

Our study was associated with some limitations. First, we only investigated the baseline level of inflammatory biomarkers; however, as previously discussed, these markers might undergo temporal changes later in the disease course and affect prognosis. Furthermore, the interpretation of our results might be limited by the small sample size, particularly in the deceased group. Thus, studies with larger sample size are warranted to investigate the relationship between dynamic changes of inflammatory markers and prognosis of patients with COVID-19.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Alireza Abrishami: Conceptualization, Methodology, Data curation, Project administration, Supervision. **Vahid Eslami:** Conceptualization, Methodology, Data curation, Supervision. **Zahra Baharvand:** Conceptualization, Methodology, Data curation, Formal analysis. **Nastaran Khalili:** Writing - original draft, Formal analysis, Investigation. **Somayeh Saghamanesh:** Software, Data curation. **Ehsan Zarei:** Data curation, Visualization. **Morteza Sanei-Taheri:** Conceptualization, Resources, Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2020.107174.

References

[1] C. Huang, Y. Wang, X. Li, et al., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, The Lancet 395 (10223) (2020) 497–506.
[2] X. Feng, S. Li, Q. Sun, et al., Immune-inflammatory parameters in COVID-19 cases: a systematic review and meta-analysis, Front. Med. 7 (2020) 301, https://doi.org/10.3389/fmed.2020.00301. PubMed PMID: 32582743; PubMed Central PMCID: PMCPMC7295898. eng.
[3] N. Ali, Elevated level of C-reactive protein may be an early marker to predict risk for severity of COVID-19, 92(11) (2020) 2409–2411. doi: 10.1002/jmv.26097.
[4] E. Poggiali, D. Zaino, P. Immovilli, et al., Lactate dehydrogenase and C-reactive protein as predictors of respiratory failure in CoVID-19 patients, Clinica Chimica Acta. 509 (2020) 135–138, https://doi.org/10.1016/j.cca.2020.06.012.
[5] B.M. Henry, G. Aggarwal, J. Wong, et al., Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: a pooled analysis, Am. J. Emerg. Med. (2020), https://doi.org/10.1016/j.ajem.2020.05.073. PubMed PMID: PMC7251362; eng.
[6] A.M. Rychter, A. Zawada, A.E. Ratajczak, et al., Should patients with obesity be more afraid of COVID-19? Obes Rev. 21 (9) (2020) e13083, https://doi.org/10.1111/obr.13083. PubMed PMID: 32583537; eng.
[7] P. Bandaru, H. Rajkumar, G. Nappanveettil, The impact of obesity on immune response to infection and vaccine: an insight into plausible mechanisms, J. Endocrinol. Metab. Synd. 2 (2) (2013) 1000113–1000122.
[8] P.M. Ryan, N.M. Caplice, Is adipose tissue a reservoir for viral spread, immune activation, and cytokine amplification in coronavirus disease 2019? Obesity. 28 (7) (2020) 1191–1194, https://doi.org/10.1002/oby.22843.
[9] A. Mahdavi, N. Khalili, A.H. Davarpanah, et al., Radiologic Management of COVID-19: preliminary experience of the Iranian society of radiology COVID-19 consultant group (ISRCC) [Letter], Iran. J. Radiol. 17 (2) (2020), e102324, https://doi.org/10.5812/iranjradiol.102324.
[10] A. Fedorov, R. Beichel, J. Kalpathy-Cramer, et al., 3D Slicer as an image computing platform for the Quantitative Imaging Network, Magn. Reson. Imaging. 30 (9) (2012) 1323–1341, https://doi.org/10.1016/j.mri.2012.05.001. PubMed PMID: 22770690; eng.
[11] U.E. Martinez-Outschoorn, M. Prisco, A. Ertel, et al., Ketones and lactate increase cancer cell "stemness," driving recurrence, metastasis and poor clinical outcome in breast cancer: achieving personalized medicine via Metabolo-Genomics, Cell cycle (Georgetown, Tex). 10 (8) (2011) 1271–1286, https://doi.org/10.4161/cc.10.8.15330. PubMed PMID: 21512313; PubMed Central PMCID: PMCPMC3117136. eng.
[12] A. Assiri, J.A. Al-Tawfiq, A.A. Al-Rabeeah, et al., Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study, Lancet. Infect. Dis 13 (9) (2013) 752–761, https://doi.org/10.1016/s1473-3099(13)70204-4. PubMed PMID: 23891402; PubMed Central PMCID: PMCPMC7185445. eng.
[13] W. Ye, G. Chen, X. Li, et al., Dynamic changes of D-dimer and neutrophil-lymphocyte count ratio as prognostic biomarkers in COVID-19, Respir Res. 21 (1) (2020) 169, https://doi.org/10.1186/s12931-020-01428-7. PubMed PMID: 32620118; eng.
[14] F. Zhou, T. Yu, R. Du, et al., Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study, Lancet. 395 (10229) (2020) 1054–1062, https://doi.org/10.1016/s0140-6736(20)30566-3. PubMed PMID: 32171076; eng.

[15] A.-P. Yang, J.-P. Liu, W.-Q. Tao, et al., The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients, Int. Immunopharmacol. 84 (2020) 106504, https://doi.org/10.1016/j.intimp.2020.106504. PubMed PMID: 32304994; eng.
[16] C.W.K. Lam, M.H.M. Chan, C.K. Wong, Severe acute respiratory syndrome: clinical and laboratory manifestations, Clin. Biochem. Rev. 25 (2) (2004) 121–132. PubMed PMID: 18458712; eng.
[17] J.-H. Ko, G.E. Park, J.Y. Lee, et al., Predictive factors for pneumonia development and progression to respiratory failure in MERS-CoV infected patients, J Infect. 73 (5) (2016) 468–475, https://doi.org/10.1016/j.jinf.2016.08.005. PubMed PMID: 27519621; eng.
[18] C. Qin, L. Zhou, Z. Hu, et al., Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China, Clin. Infect. Dis. 71 (15) (2020) 762–768, https://doi.org/10.1093/cid/ciaa248. PubMed PMID: 32161940; eng.
[19] A. Słomka, M. Kowalewski, E. Żekanowska, Coronavirus disease 2019 (COVID-19): a short review on hematological manifestations, Pathogens. 9 (6) (2020) 493, https://doi.org/10.3390/pathogens9060493. PubMed PMID: 32575786; eng.
[20] B. Cheng, J. Hu, X. Zuo, et al., Predictors of progression from moderate to severe COVID-19: a retrospective cohort, Clin. Microbiol. Infect.: Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis. (2020), https://doi.org/10.1016/j.cmi.2020.06.033. PubMed PMID: 32622952; PubMed Central PMCID: PMCPMC7331556. eng.
[21] M. Fei, F. Tong, X. Tao, et al., Value of neutrophil-to-lymphocyte ratio in the classification diagnosis of coronavirus disease 2019, Zhonghua wei zhong bing ji jiu yi xue. 32 (5) (2020) 554–558, https://doi.org/10.3760/cma.j.cn121430-20200413-00506. PubMed PMID: 32576346; chi.
[22] F.A. Lagunas-Rangel, Neutrophil-to-lymphocyte ratio and lymphocyte-to-C-reactive protein ratio in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis, J. Med. Virol. (2020), https://doi.org/10.1002/jmv.25819. PubMed PMID: 32242950; PubMed Central PMCID: PMCPMC7228336. eng.
[23] J. Fu, J. Kong, W. Wang, et al., The clinical implication of dynamic neutrophil to lymphocyte ratio and D-dimer in COVID-19: a retrospective study in Suzhou China, Thromb. Res. 192 (2020) 3–8, https://doi.org/10.1016/j.thromres.2020.05.006. PubMed PMID: 32407937; PubMed Central PMCID: PMCPMC7201241. eng.
[24] G. Bomhof, P. Mutsaers, F.W.G. Leebeek, et al., COVID-19-associated immune thrombocytopenia, Br. J. Haematol. 190 (2) (2020 Jul) e61–e64, https://doi.org/10.1111/bjh.16850. PubMed PMID: 32420612; PubMed Central PMCID: PMCPMC7276755. eng.
[25] G. Lippi, M. Plebani, B.M. Henry, Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a meta-analysis, Clin. Chim Acta. 506 (2020) 145–148, https://doi.org/10.1016/j.cca.2020.03.022. PubMed PMID: 32178975; PubMed Central PMCID: PMCPMC7102663. eng.
[26] R. Qu, Y. Ling, Y.H. Zhang, et al., Platelet-to-lymphocyte ratio is associated with prognosis in patients with coronavirus disease-19, J. Med. Virol. (2020), https://doi.org/10.1002/jmv.25767. PubMed PMID: 32181903; PubMed Central PMCID: PMCPMC7228291. eng.
[27] A. Chait, L.J. den Hartigh, Adipose tissue distribution, inflammation and its metabolic consequences, including diabetes and cardiovascular disease, Front. Cardiovasc. Med. 7 (2020) 22, https://doi.org/10.3389/fcvm.2020.00022. PubMed PMID: 32158768; PubMed Central PMCID: PMCPMC7052117. eng.
[28] M.D. Van Kerkhove, K.A. Vandemaele, V. Shinde, et al., Risk factors for severe outcomes following 2009 influenza A (H1N1) infection: a global pooled analysis, PLoS Med. 8 (7) (2011), e1001053, https://doi.org/10.1371/journal.pmed.1001053. PubMed PMID: 21750667; PubMed Central PMCID: PMCPMC3130021 eng.
[29] H.E. Maier, R. Lopez, N. Sanchez, et al., Obesity increases the duration of influenza A virus shedding in adults, J. Infect. Dis. 218 (9) (2018) 1378–1382, https://doi.org/10.1093/infdis/jiy370. PubMed PMID: 30085119; PubMed Central PMCID: PMC6151083. eng.
[30] F. Briand-Mésange, S. Trudel, J. Salles, et al., Possible role of adipose tissue and the endocannabinoid system in coronavirus disease 2019 pathogenesis: can rimonabant return? Obesity (Silver Spring, Md). 28 (9) (2020) 1580–1581, https://doi.org/10.1002/oby.22916. PubMed PMID: 32463562; eng.
[31] I.L. Kruglikov, P.E. Scherer, The role of adipocytes and adipocyte-like cells in the severity of COVID-19 infections, Obesity (Silver Spring, Md). 28 (7) (2020) 1187–1190, https://doi.org/10.1002/oby.22856. PubMed PMID: 32339391; PubMed Central PMCID: PMCPMC7267593. eng.
[32] H. Zbinden-Foncea, M. Francaux, L. Deldicque, et al., Does high cardiorespiratory fitness confer some protection against proinflammatory responses after infection by SARS-CoV-2? Obesity (Silver Spring, Md). 28 (8) (2020) 1378–1381, https://doi.org/10.1002/oby.22849. PubMed PMID: 32324968; PubMed Central PMCID: PMCPMC7264673. eng.
[33] A.H. Talman, P.J. Psaltis, J.D. Cameron, et al., Epicardial adipose tissue: far more than a fat depot, Cardiovasc. Diagn. Therapy. 4 (6) (2014) 416–429, https://doi.org/10.3978/j.issn.2223-3652.2014.11.05. PubMed PMID: 25610800; PubMed Central PMCID: PMCPMC4278038. eng.
[34] M. Deng, Y. Qi, L. Deng, et al., Obesity as a potential predictor of disease severity in young COVID-19 patients: a retrospective study, Obesity (Silver Spring, Md). 28 (10) (2020) 1815–1825, https://doi.org/10.1002/oby.22943. PubMed PMID: 32602202; eng.
[35] G. Iacobellis, F. Secchi, G. Capitanio, et al., Epicardial Fat Inflammation in severe COVID-19, Obesity. (2020), https://doi.org/10.1002/oby.23019.
[36] M.A. Derya, V. Demir, H. Ede, Relationship between neutrophil/lymphocyte ratio and epicardial fat tissue thickness in patients with newly diagnosed hypertension, J. Int. Med. Res. 46 (3) (2018) 940–950, https://doi.org/10.1177/0300060517749130. PubMed PMID: 29332485; PubMed Central PMCID: PMCPMC5972270. eng.
[37] E. Akıl, M.A. Akıl, S. Varol, et al., Echocardiographic epicardial fat thickness and neutrophil to lymphocyte ratio are novel inflammatory predictors of cerebral ischemic stroke, J. Stroke Cerebrovasc. Dis.: Off. J. Natl. Stroke Assoc. 23 (9) (2014 Oct) 2328–2334, https://doi.org/10.1016/j.jstrokecerebrovasdis.2014.04.028. PubMed PMID: 25200242; eng.
[38] A.G. Ertem, M. Erdogan, C. Koseoglu, et al., Epicardial fat tissue thickness is increased in patients with lichen planus and is linked to inflammation and dyslipidemia, Revista Portuguesa de Cardiologia. 35 (10) (2016) 525–530, https://doi.org/10.1016/j.repc.2016.04.010.
[39] E.M. Akbas, H. Hamur, L. Demirtas, et al., Predictors of epicardial adipose tissue in patients with type 2 diabetes mellitus, Diabetol. Metabolic Syndrome. 6 (2014) 55, https://doi.org/10.1186/1758-5996-6-55. PubMed PMID: 24822086; PubMed Central PMCID: PMCPMC4018267. eng.